

832-Pos Board B601**Electrophysiology and Single Cell Reverse Transcription Quantitative PCR in GnRH Neurons**

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Release of gonadotrophin-releasing hormone (GnRH) from specialized hypothalamic neurons is the final common pathway in the endocrine reproductive axis and plays an important role in reproductive health. Regular and timely electrical activity of GnRH neurons accompanies the pulsatile release of GnRH that is plays a central role in release of luteinizing hormone and follicle stimulating hormone required for normal fertility in all vertebrates. Understanding the molecular basis of the neuronal electrical activity is critical to understanding excitation-release coupling in GnRH neurons. Despite the fundamental importance of GnRH neurons in the reproductive axis, little is known about the molecular basis of electrical activity in GnRH neurons. GnRH neurons are spontaneously active, and pacemaking potentials have been identified in GnRH neurons. We used transgenic rats expressing GFP driven by a GnRH promoter to identify individual GnRH neurons. We performed electrophysiology and single cell quantitative RT-PCR to determine the electrophysiology and molecular identity of ion channels in isolated GFP-labeled GnRH neurons isolated from brains of regularly cycling female rats. We have identified Kv4.3 transcripts, suggesting the presence of a transient outward-type current (I_A). We also identified a hyperpolarization-activated current that is probably encoded by HCN2 and/or HCN3 and may be responsible for the pacemaking activity critical to GnRH neuronal function.

833-Pos Board B602**TRPV4 and KCa: Modelling the Perfect Couple?**

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The hypothalamus is responsible for maintaining body fluid osmolarity within a narrow range (~290-300mOsm) [1]; in particular the paraventricular nucleus (PVN) is thought to have a key role in osmoregulation. Previously we have shown changes in action current frequency upon hypotonic challenge within the PVN using patch-clamp electrophysiology [2], with evidence suggesting these changes are due to the activation of the transient receptor potential vanilloid 4 (TRPV4) and calcium-activated potassium (KCa) channels. Our data supports a functional coupling between the TRPV4 and KCa channels, leading us to the hypothesis that upon hypotonic challenge TRPV4 activates KCa through influx of Ca^{2+} , leading to an efflux of K^+ , as shown in other cells [3] leading to hyperpolarisation of the cell. This hyperpolarisation itself is suggested to create a positive feedback loop increasing the driving force for Ca^{2+} entry [4]. To investigate this hypothesis we have developed our existing NEURON model (University of Yale) [5], modelling the action of TRPV4 and KCa. In the model decreased osmolarity caused action potential (AP) frequency reduction, with a $93 \pm 3\%$ decrease of AP frequency at 290mOsm and a half maximum of 304 ± 0.4 mOsm, dependant on starting parameters. Block of TRPV4 or KCa channels prevented this effect with AP frequency remaining at $100 \pm 0\%$ regardless of osmolarity. To test positive feedback we simulated TRPV4 activity and measured simulated TRPV4 Ca^{2+} current with and without the presence of KCa activity. Breaking the positive feedback loop by block of KCa, as predicted, significantly reduced TRPV4 Ca^{2+} current by 640 ± 0.1 pA/s.cm² ($n=7$; $p<0.005$).

This model, together with our previous data provides further evidence for a functional coupling between TRPV4 and KCa channels within neurones in the PVN, supporting our hypothesis of a positive feedback system.

834-Pos Board B603**Modeling Calcium Diffusion in Chondroitin Sulfate using a Bimolecular Reaction Scheme**

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 Ca^{2+} diffusion in brain extracellular space (ECS) regulates local Ca^{2+} concentration and influences synaptic transmission and neuronal excitability. Ca^{2+} diffusion is controlled by ECS geometry and extracellular matrix, a major component of which is chondroitin sulfate (CS). Previous experiments (Hrabetova et al., *J. Physiol.* 2009, 587:4029) show that Ca^{2+} movement is reduced when interacting with CS, however the process still obeys the diffusion equation. This implies that Ca^{2+} -CS interaction can be modeled as a fast equilibrium bimolecular reaction (FEBR; Nicholson et al. *Comput. Visual Sci.*, 2012, 10.1007/s00791-012-0185-9). The FEBR would provide an alternative to solving the Poisson-Boltzmann equation for electrostatic interactions between negatively charged CS and mobile cations.

Our objectives were 1) to test whether the FEBR model describes previous experimental data, 2) if so, to determine the dissociation constant K_d , (ratio between backward and forward rate constants), 3) to explore the effect of background Ca^{2+} and/or Na^+ on Ca^{2+} diffusion and 4) use a Monte Carlo simulator (*MCell*; www.mcell.org) to verify results.

We developed analytical expressions for the Ca^{2+} effective diffusion coefficient in the presence of CS and compared results to experimental data with different background Ca^{2+} and CS concentrations (Magdelenat et al., *Biopolymers*, 1974, 13:1535; Maroudas et al., *Biophys. Chem.*, 1988, 32:257). This validated the FEBR approach and provided estimates of K_d (0.01 to 0.1 mM) in agreement with literature data. K_d values were resolved into forward and backward rate constants using the Damköhler formula and the results further tested with *MCell*. Finally, we extended the work to include additional background Na^+ .

We conclude that the FEBR model captures the main features of Ca^{2+} diffusion in CS matrix and can be extended to interactions involving multiple cations. Supported by NIH/NINDS grant R01-NS-28642.

835-Pos Board B604**The Delicate Bistability of CaMKII Activation**

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Calcium/calmodulin-dependent protein kinase II (CaMKII) is one of the most abundant proteins in the brain and is essential for learning and memory. The activity of CaMKII is regulated both by calmodulin binding and by phosphorylation on Thr286, which maintains the kinase in a partially active state even in the absence of a calcium/calmodulin signal, thus prolonging the effects of transient calcium signaling. CaMKII exists as a 12 subunit holoenzyme, and phosphorylation at Thr286 occurs by an intra-holoenzyme, inter-subunit reaction. An autophosphorylating kinase, in conjunction with a phosphatase, can potentially form a bistable switch, and it has been proposed that bistability in the CaMKII system may constitute the biochemical change underlying long-term memory. Previous modeling efforts have suggested that bistability is likely under physiological conditions, but experimental studies have proved inconclusive. Previous modeling efforts involved several significant approximations in order to overcome the combinatorial complexity inherent in a multi-subunit, multi-state system. Here we develop a stochastic, particle-based model of CaMKII dynamics and activation which naturally avoids the issues of combinatorial complexity, and thus allows us to study an exact model without resorting to severe approximations. We find that bistability is possible, but for any reasonable choice of parameters bistability only occurs at calcium concentrations much higher than basal calcium levels, and then only over a very narrow range of calcium concentration. We conclude that bistability is not a physiologically relevant feature of the CaMKII system, which should appear to behave as an ultra-sensitive switch. On the other hand, the system dynamics are generally very slow. Transiently activated kinase can maintain its activity over the time scales of many of the published experimental protocols, which may account for the conflicting reports of kinase bistability. This work was supported by NIH grants 1F32-NS077751-01 and P41GM103313.

836-Pos Board B605**Spatiotemporal Dynamics of Calmodulin in Dendritic Spines during Calcium Influx**

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Experiments by Lee et al. (Lee et al., 2009) provide compelling evidence of spatially inhomogeneous reactant distributions in dendritic spines. We have built a reaction-diffusion model of a dendritic spine using the calmodulin activation kinetics developed by Faas et al. (Faas et al., 2011). Upon calcium influx from voltage-sensitive calcium channels, we find spatial inhomogeneities lasting 2 ms in the distribution of calmodulin with N-lobe-bound calcium, but no significant inhomogeneities in the distributions of calmodulin with C-lobe-bound calcium or calbindin. In the presence of 5 mM EGTA, the spatial inhomogeneity in the concentration of calmodulin with N-lobe-bound calcium persists for 1 ms, eventually becoming restricted to the outer 200 nm of the spine at 20 mM EGTA. Calmodulin with C-lobe-bound calcium continues to penetrate to the interior, but at a lower concentration and with an inhomogeneous distribution. In the presence of 5 mM and 20 mM BAPTA, concentrations of both species drop sharply and they are not able to diffuse to the interior of the spine. We believe that these spatial inhomogeneities (arising during calcium influx) can assist in explaining the results presented by Lee et al. Furthermore, our work forms a basis for a spatially-resolved reaction-diffusion model of dendritic spines that includes the reactions underlying the activation,